Airborne enzyme measurements to detect indoor mould exposure

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Mould in buildings constitutes a threat to health. Present methods to determine the moulds comprise counting of spores or determination of viable moulds which give imprecise measures of total mould cell biomass. Analysis of ergosterol and β-glucan as markers of mould cell biomass is expensive and cumbersome. To evaluate if airborne enzyme activity was related to mould in buildings air samples were taken using an impinger technique or cellulose filters in 386 rooms in 141 buildings. The samples were analysed for the activity of *N*-acetylhexosaminidase (NAHA) and expressed as enzyme units per m^3 (EU per m^3). The highest value found in a building was used for the classification of the building and was related to the results from the subsequent technical inspection. In buildings without mould damage, the NAHA activity was generally below 20 EU per m^3. In buildings with mould damage, almost all the buildings had activities above 20 EU per m^3 (specificity 85%). At 30 EU per m^3 the specificity was 100%. Measurements of airborne enzyme activity have a high sensitivity and specificity to identify buildings with mould problems. The method can be used in the investigations of building related symptoms or for home exposure characteristics when investigating diseases such as asthma that can be related to mould exposure.

Introduction

Indoor mould contamination is an important health issue and exposure to elevated levels of airborne moulds may cause a number of unspecific symptoms such as swollen nasal passages, airways irritation, fatigue and headache.[1–5] At high levels there is also a risk for hypersensitivity pneumonitis[6,7] and sarcoidosis.[8] In view of this, methods to detect mould in buildings are important, both for preventive purposes and for surveillance in connection with flooding or other water damage.[9]

Conventional methods to detect the presence of mould indoors are visual inspection and sampling of air or surfaces with subsequent microscopic evaluation or growth on agar plates. The determination of microbial cell wall agents (MCWAs) such as ergosterol, β-glucan, and phospholipid fatty acid has also been used.[10–13]

Another MCWA is the enzyme β-*N*-acetylhexosaminidase (NAHA). Previous studies have reported that NAHA represents fungal biomass both in the growth and stationary phase.[14,15] Significant correlations between NAHA and total spore counts were found in air samples and in dust generated from biomass in a biofuel plant.[16,17] Strong correlations were found between fungal biomass (gravimetric weight) and NAHA in fungal species grown on nutrient agar and between ergosterol and NAHA activity on mould contaminated gypsum boards.[18] A linear correlation between NAHA and *Aspergillus niger* biomass has been reported.[19] These studies demonstrate that NAHA can be used as a marker for mould cell biomass. Fluorometric detection of the NAHA activity is rapid, not cumbersome and can be performed on site.

The purpose of the present study was to assess whether airborne sampling of NAHA activity could be used to distinguish between buildings with and without mould damage. Air samples were taken in different rooms in buildings which were later inspected for mould damage.

Material and methods

Buildings

Buildings for the study were recruited by advertising in local newspapers. Those who responded wanted to know if mould was present as they had experienced odour or observed water

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Environmental impact

Fungi in the indoor environment are a major risk factor for several diseases, particularly asthma. From diagnostic and prevention points of view, methods that describe the exposure are important. Fungal cell walls contain a number of substances that have immunological properties and the determination of total cell mass is thus preferable from a toxicological point of view. With the enzyme method described in the manuscript, it is possible to identify the airborne fungal cell mass exposure indoors with a high specificity and sensitivity. Enzyme measurements can be used to identify buildings with fungi in the indoor environment or in the investigations of respiratory disease, related to the indoor environment.

damage. Others wanted to ensure that the remedy actions previously undertaken for mould damage had given the expected results and yet others were just curious to know if mould could be found in their houses (as the testing was without charge).

Airborne samples were taken in 2–4 rooms in each building and when the tenants indicated a suspect source, samples were taken in that room and in the adjacent room. A total of 386 rooms in 141 buildings was investigated.

Within 1–2 weeks after the air sampling, all buildings were inspected for the presence of moulds by the same experienced damage assessor. The inspection included recording of location, severity and extension of mould damage, details on construction, particularly ground characteristics and history of flooding and other water damage. Based on this information the building was classified into no mould damage/mould damage present. The assessor was unaware of the air sampling results.

Air sampling

In the first part of the study the amount of airborne NAHA was not known and thus a high volume air sampler was used (a liquid impinger, OMNI 3000, Evogen Inc., Kansas City, MI, USA). The equipment was placed on the floor in the rooms investigated with the air intake 15 cm above the floor. The sampler was run for 10 minutes with a sampling volume of 300 L min^{-1}. During sampling, 10 mL sterile water from a liquid capsule are rotated inside a glass cylinder and the air passes through the water which traps the particles. At the end of the sampling session, the water is pumped back into the capsule and used for the analysis. Impinger sampling was performed in 184 rooms in 72 buildings.

In the second part of the study the air samples were taken using cellulose acetate filters in a sampling cassette (PCM Cassettes preloaded with mixed Cellulose Ester filters, 25 mm, 0.8 μm pore size, Zefon International, Inc., Ocala, FL, USA). Based on the experience from the liquid impinger the sampling with the filter was set at 300 L air for 15 minutes at 40 cm above the ground with an open-faced cassette. Filter sampling was performed in 202 rooms in 69 buildings.

To ensure representative indoor conditions, all doors and windows in the rooms sampled were closed for at least 5 hours, usually from the evening, before sampling and all mechanical ventilation was switched off.

Enzyme analysis

Two mL of the water from the OMNI capsule were filtered through a membrane filter (Millipore, Millex-GP, 0.22 μm) and 2.5 mL of a fluorogenic enzyme substrate (4-methylumbelliferyl *N*-acetyl-β-D-glucosaminide, Mycometer A/S, Copenhagen, Denmark) were added to the filter. The standard reaction time was 60 minutes at 23 °C. At higher or lower ambient temperatures, the reaction time was adjusted according to a set scheme of times according to the manufacturer's recommendation and based on the Arrhenius equation from measurements of NAHA activity from a number of fungi at different temperatures.

Thereafter the filters were flushed with two mL of an alkaline buffer (the developer) which was collected in a cuvette. The fluorescence of this fluid was read in a fluorometer (Picofluor, Turner Designs, Sunnyvale, CA, USA) and the fluorescence value for a control filter was deducted from the sample value. One count is equal to 2.3 ng *Aspergillus oryzae*. To obtain a more robust measure and avoid non-informative figures, the values read in the fluorometer were divided by 10 and given as a round figure to express the NAHA enzyme activity in units (EU per m^3).

To analyse the cassette filters one mL of the enzyme substrate (Mycometer A/S) was added to the filter and incubated for around 30 minutes—the exact time being set by the room temperature. Thereafter two mL of an alkaline buffer (the developer) were added to the filter and the liquid in the filter holder was sucked out through the filter and collected in a cuvette. The fluorescence of this fluid was read as for the OMNI sampling.

Definition of NAHA indication of mould damage

During the project it was found that there was a significant relation between the distance to the site of damage (number of rooms apart) and the enzyme activity in buildings where mould damage was identified ($r^2 = -0.500$, $p = 0.01$, Spearman's test). For this reason, the highest measured NAHA value within a building was used as an operational classification of the building, prior to the technical mould inspection.

Statistical analysis

Comparisons between buildings classified as with or without mould were made using non-parametric testing. Relations between the values from repeated samples were evaluated using correlation analysis.

Results

To test the reproducibility of sampling with cassette filters samples were taken in parallel in 25 different rooms. The R^2 was 0.956 with ANOVA, f 504.2, $p < 0.001$. Samples were also taken on two occasions a few weeks apart in 26 rooms. The R^2 was 0.796 with ANOVA, f 93.42, $p < 0.001$. Repeated readings of the same sample on the fluorometer showed very small variations in the order of a few units.

The NAHA values in relation to the presence of mould damage are reported in Table 1.

The NAHA levels were significantly higher in buildings with mould damage ($p < 0.001$, Mann–Whitney).

Table 1 Airborne NAHA values (EU per m^3) in buildings with and without mould damage

Sampling method	Impinger		Filter	
Mould damage	No	Yes[a]	No	Yes[a]
n	44	28	27	42
Mean	20.2	55.1	13.3	41.9
Median	16.9	57.2	14.0	31.9
SD	8.8	23.4	7.5	26.3
SEM	1.3	4.4	1.4	3.2

[a] Significantly different from "no mould", $p < 0.001$, Mann–Whitney.

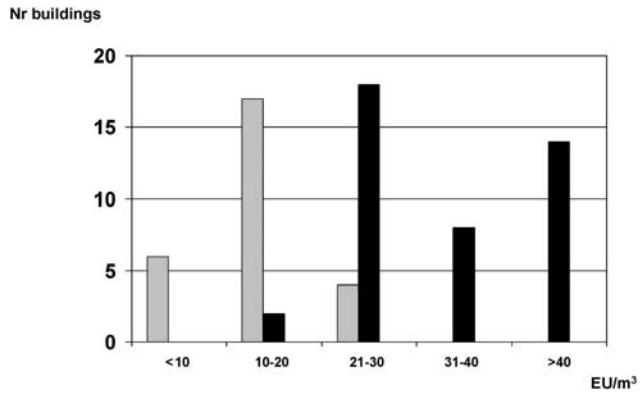

Fig. 1 Enzyme activities (NAHA EU per m^3) in buildings without (grey) and with (black) mould damage (filter sampling).

The distribution of the highest NAHA activity on filters collected from buildings with or without mould damage is illustrated in Fig. 1.

At a cut off value of 20 NAHA EU per m^3, mouldy buildings were identified with a sensitivity of 95% and a specificity of 85%. At 30 EU per m^3 the sensitivity was 55% and the specificity 100%.

Discussion

The major result from the study is that the highest measured airborne NAHA activity in a building, irrespectively of the air sampling technique, was highly related to the presence of mould damage and that mouldy buildings could be identified with a high sensitivity and specificity.

There are some methodological issues to consider. The measures were made on one occasion but this is representative for the situation in field investigations. There was a very small variation between samples taken in parallel and a relatively good relation between samples taken at different times. The number of rooms sampled in the different buildings was limited to two and at most three. Non-systematic repeated measures within the project as well as experience from other studies[20] suggest, however, that activities in a certain room vary relatively little within a year, particularly in buildings with high levels of moulds.

The air sampling was performed using two different devices. The high volume sampler was chosen as there was no information available at the start of the study on the amount of NAHA in the air. Subsequently it was shown that sampling with a filter cassette gave a sufficient amount of NAHA. From a practical point of view sampling with the filter cassette is preferable and the equipment required is fairly inexpensive. For this reason it was not considered to be of interest to evaluate in detail the small differences in values obtained with the high volume sampler and the cassette filter.

A number of previous studies reviewed in the Introduction have demonstrated that NAHA is a valid measure of fungal biomass. It is known that fragments of fungal spores and cells comprise a significant part of the total exposure.[21] As NAHA measures the enzyme activity in the fragments as well, it is a suitable method from toxicological and risk assessment points of view.

In the evaluation of the enzyme values, the highest recorded value in a building was taken as the operational definition of the building status. This measure was a good predictor for the presence of mould damage. It is well-known that mould damage in a building is often located at a specific site and that the inhabitants often report symptoms in relation to a specific room. The presence of a high value in one room as compared to adjacent rooms could thus be useful for a further technical exploration even if the value itself does not exceed 30 EU per m^3.

The enzyme activities read on the air sampling filter represent the reaction between the enzyme and a reagent. The value read depends on the incubation time, the amount of substrate used and the temperature. The values obtained, using the impinger method, were slightly higher than the values obtained with the filter sampling. This could be due to the differences in the collection method—one method samples in a liquid medium and the other under dry conditions. From an application point of view the specificity/sensitivity values were almost the same and both methods thus give relevant results. Different values for the enzyme activity could be obtained if other methods for collection of the samples or other analysis procedures were used. The relative difference between buildings with and without mould problems will, however, remain as illustrated by the very similar outcomes of the impinger and filter sampling. From a practical point of view the filter sampling is the method of choice.

The analysis of airborne samples comprised the determination of NAHA as a measure of mould biomass. Among fungal species, NAHA is produced mainly by filamentous fungi *e.g.* Aspergillus species. NAHA is produced by a range of organisms including bacteria, fungi, protozoa, and mammalian cells and is thus not specific for fungi. On the other hand when the fungi grow indoors such as in humid conditions, there is no reason to believe that other sources of NAHA such as pollen or mammalian cells would also multiply. The specificity for detection of buildings with moulds in this study was very high and the possible confounding factors thus did not play an important role. From a practical point of view NAHA can thus be considered a good indicator of fungi in indoor conditions with increased humidity.

A few of the buildings with mould damage had low enzyme activities. It is likely that some locations of mould damage within buildings will not give rise to an increased airborne exposure in an adjacent room. In such cases a low enzyme activity would represent a false negative result. From a practical application point of view this means that determinations of airborne enzyme activities are of value particularly in buildings with high levels. At activities above 30 EU per m^3 the specificity of the enzyme method was 100%.

In conclusion, the results from this investigation suggest that measurements of airborne NAHA activity are a useful tool for the detection of mould damage and for the identification of buildings with mould damage with a high specificity and a high sensitivity. The method can be used in investigations on building related symptoms and when investigating diseases that are related to mould exposure such as asthma.

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